*Institute Report No. 407*

# Acute Dermal Toxicity of DIGL-RP Solid Propellant in Rabbits

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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY



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Toxicology Series: 162



LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

**Acute Dermal Toxicity of DIGL-RP Solid Propellant in Rabbits (Toxicology Series 162)-- Brown *et al.***

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Donald G. Corby 30 Oct 89

**Donald G. Corby** **(date)**
**COL, MC**
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The acute dermal toxicity of DIGL-RP Solid Propellant was evaluated in six male and six female New Zealand White rabbits. Moistened ground DIGL-RP (2 g/kg) was applied topically to the clipped dorsal skin surface under a semi-occlusive wrap for 24 hours. No signs of dermal irritation, or systemic toxicity, or death were obtained that could be attributed to DIGL-RP. These data indicate that DIGL-RP Solid Propellant does not produce systemic toxicity when administered by 24-hour topical application at a limit dose of 2 g/kg.

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## ABSTRACT

The acute dermal toxicity of DIGL-RP Solid Propellant was evaluated in six male and six female New Zealand White rabbits. Moistened ground DIGL-RP (2 g/kg) was applied topically to the clipped dorsal skin surface under a semi-occlusive wrap for 24 hours. No signs of dermal irritation, or systemic toxicity, or death were obtained that could be attributed to DIGL-RP. These data indicate that DIGL-RP Solid Propellant does not produce systemic toxicity when administered by 24-hour topical application at a limit dose of 2 g/kg.



KEY WORDS: Acute Dermal Toxicity, DIGL RP Solid Propellant, Diethyleneglycol Dinitrate, Munition, Rabbit, Mammalian Toxicology

## PREFACE

TYPE REPORT: Acute Dermal Toxicity GLP Report

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US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Develcpment Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
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GLP STUDY NUMBER: 85024

STUDY DIRECTOR: Don W. Korte, Jr., PhD, LTC, MSC
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PRINCIPAL INVESTIGATOR: Larry D. Brown, DVM, LTC, VC, Diplomate
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CO-INVESTIGATOR: James D. Justus, MPA, SSG

PATHOLOGIST: G. Tracy Makovec, DVM, MAJ, VC, Diplomate
American College of Veterinary Pathologists

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: DIGL-RP Solid Propellant

INCLUSIVE STUDY DATES: 14 Nov 1985 - 18 Dec 1985

OBJECTIVE:

The objective of this study was to evaluate the acute dermal toxicity of DIGL-RP Solid Propellant in male and female New Zealand White rabbits.

## ACKNOWLEDGMENTS

SP4 James J. Fischer, and SP4 Scott L. Schwebe, SP4 Gayle Orner, SP4 Theresa L. Polk, Obie Goodrich and Diane Arevalo assisted in conducting this research; SP4 John Ryabik, BS and SP4 Paul B. Simboli, BS, provided chemical preparation and analysis; Colleen S. Kamiyama and Ann L. Wilkinson provided secretarial assistance.

## SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 85024 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte, Jr. 4 OCT 89

DON W. KORTE, JR., PhD / DATE
LTC, MSC
Study Director

Larry D. Brown 13 July 1989

LARRY D. BROWN, DVM / DATE
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DAC
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PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA 26 October 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85Ø24

1. This is to certify that the protocol for LAIR GLP Study 85Ø24 was reviewed on 1Ø May 1985.

2. The institute report entitled "Acute Dermal Toxicity of DIGL-RP Solid Propellant in Rabbits," Toxicology Series 162, was audited on 25 October 1989.

Carolyn M. Lewis

CAROLYN M. LEWIS
Diplomate, American Board of
Toxicology
Quality Assurance Auditor

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## INTRODUCTION

The Department of Defense is considering the use of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in new propellant formulations. However, considerable gaps in the toxicology data of the compounds were identified during a review of their health effects (1) conducted for the US Army Biomedical Research and Development Laboratory (USABRDL). Consequently, USABRDL has tasked the Division of Toxicology, Letterman Army Institute of Research (LAIR), to conduct an initial health effects evaluation of the proposed replacement nitrate esters. This initial evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP, includes the Ames mutagenicity assay, acute oral toxicity tests in rats and mice, acute dermal toxicity studies in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs.

### Objective of Study

The objective of this study was to determine the acute dermal toxicity of DIGL-RP Solid Propellant in male and female New Zealand White rabbits.

## MATERIALS

### Test Substance

Chemical Name: DIGL-RP Solid Propellant

LAIR Code Number: TP57

Lot Number: RAD83M001S169

Description: Solid black cylinders (stick configuration)

Other test substance information is presented in Appendix A.

Vehicle

The vehicle was sterile isotonic saline (Viaflex® Sodium Chloride Injection, USP; Travenol Laboratories, Inc., Deerfield, IL 60015, Lot No. 3C979X6, Exp. Date June 1986).

Animal Data

Six male and seven female young New Zealand White rabbits (Elkhorn Rabbitry, Watsonville, CA) from a shipment that arrived at LAIR on 14 November 1985 were assigned to the study. The 13 rabbits were identified individually by ear tattoos. One rabbit (85F292) in the shipment was submitted for necropsy quality control on 18 November 1985. The animal weights ranged from 2.1 to 2.7 kg on the day after receipt and from 2.5 to 3.1 kg the day before dosing. Additional animal data appear in Appendix B.

Husbandry

The rabbits were housed individually in stainless steel wire mesh cages in racks equipped with automatic flushing dumptanks. No bedding was used in any of the cages. Water was provided *ad libitum* by continuous drip from a central line. The diet consisted of approximately 150 g per day of Purina Certified Rabbit Chow® No. 5322 (Ralston Purina Company, St. Louis, MO). The animal room temperature was maintained at 15.6°C to 21.1°C and the relative humidity was maintained at 30% to 70%, except for spikes to 90% during room cleaning. The photoperiod was 12 hours of light per day.

## METHODS

This study was performed in accordance with LAIR Standard Operating Procedure OP-STX-30, "Acute Dermal Toxicity Study" (2), and Environmental Protection Agency guidelines (3).

Acclimation/Group Assignment

Study rabbits were quarantined by the Division of Animal Care and Services, LAIR, for two weeks before being certified healthy by a staff veterinarian. During quarantine the rabbits were given one application of Canex®/mineral oil (Pitman-Moore, Inc., Washington Crossing, NJ) for ear mite protection. After being certified healthy, the rabbits were transfered to the Toxicology Suite for the remainder of the study. Randomization for group assignment was unnecessary as there was only one dose level for each sex.

Dose Levels

A "limit test" was conducted in which 6 male and 6 female rabbits received 2.0 g/kg of DIGL-RP applied as a saline paste topically to the dorsum (skin over back).

Compound Preparation

The compound (5.14 - 6.22 g, depending on animal weight) was mixed with 5 ml of isotonic saline to form a paste.

Chemical Analysis of DIGL-RP

Analysis for the DEGDN component of the DIGL-RP formulation indicated that DIGL-RP was 38.5% DEGDN, which was consistent with the 36.7% ± 1.5% value reported by the manufacturer (Appendix A).

Test Procedures

The application sites on the dorsal and lateral sections of the animals (surface area approximately 300 $cm^2$) were close-clipped with electric clippers (Oster® Model A5, Size 40 blade, Sunbeam Corp, Milwaukee, WI) 48 hours and again 24 hours before applying the test compound. The animals were weighed the day before dosing, and the quantity of compound required to provide the 2.0 g/kg limit dose was weighed. The test compound was evenly distributed over the surface of an 8 x 8 in. piece of saline-moistened gauze dressing (Topper® Gauze Sponges, Johnson & Johnson Products, Inc., New

Brunswick, NJ) which was then taped to the animal's back with hypoallergenic tape (Durapore® Surgical Tape, 3M Corp, St. Paul, MN). The trunk of the animal was then wrapped with Vet Wrap® tape (Animal Care Products, 3M Corp, St. Paul, MN) to hold the compound in place and prevent the animal from ingesting the compound. The Vet Wrap® was anchored in place cranially and caudally by strips of Elastoplast® tape (Belersdorf Co., Norwalk, CT). The patch and wrappings were left in place for 24 hours. No restraint of the animals was used except during the wrapping procedure. When the wrappings and patch were removed, the exposed area was gently wiped with a piece of saline-moistened gauze to remove any remaining test compound.

Observations

Observations for mortality and signs of acute toxicity were performed 2, 4, and 5 hours after dosing and daily for the remainder of the study according to the following procedure: (1) animals were observed undisturbed in their cages, (2) animals were removed from their cages and given a physical examination, and (3) animals were observed after being returned to their cages. A second "walk through" observation was performed each day, with only significant observations recorded. The exposed area was examined and scored 1/2 hours after patch removal and daily for the duration of the study. All lesions were noted and graded as described below. Animals were weighed weekly during the study test period.

During evaluation of the exposure site, area and intensity of each dermal reaction were graded. Grading was performed according to a scale which included five categories to describe area and four categories to describe severity. Area categories were 0 - 5%, > 5 - 10%, > 10 - 25%, > 25 - 50% and > 50%; severity was defined as slight, mild, moderate, and severe.

Necropsy

All study animals were submitted for necropsy. Those that survived the 14-day study period were necropsied immediately after being given an overdose of sodium pentobarbital followed by exsanguination from severed axillary vessels. Skin was taken from exposed and control areas of five animals and examined microscopically.

Duration of Study

The study period was 14 days and was preceded by a 19-day quarantine/acclimation period. Historical study events are listed in Appendix C.

Changes/Deviations from Protocol

All phases of this study were accomplished according to the protocol and applicable amendments, with the following exceptions: Animals were scored as described above using four categories for severity instead of the five described in SOP OPS-STX-30. This standardized the dermal scoring with scoring criteria for the dermal irritation study which also uses four categories of severity, thus minimizing confusion for the scorers. Animals were weighed one day earlier than described in the protocol, on 3 and 10 December, to coincide with clipping of the animals. It is believed that these changes did not adversely affect the outcome of the study.

Raw Data and Final Report Storage

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

## RESULTS

Twenty-four hour dermal exposure to DIGL-RP at a limit dose of 2.0 g/kg produced no mortality in the 12 rabbits evaluated in the study. During the course of the study, observations were split into two major categories: systemic (general health of the animal) and dermal.

Systemic: All 12 rabbits were observed panting during the 24-hour period they were wrapped; one male (85F295) was also observed panting on day 8; two females (85F288, 85F293) and three males (85F297, 85F299, 85F300) were observed with loose or soft stools or pasted stools on the tail on day 0 or day 1; one female (85F290) was observed with tearing in the left eye on day 0; one male (85F295) was observed with a greenish-yellow nasal discharge on day 0; and one female (85F294) was observed with wet fur from a malfunctioning watering valve on day 0. During the remainder of the study, all animals exhibited normal clinical behavior signs. None of the clinical systemic signs were interpreted as signs of toxicity attributable to DIGL-RP. The rabbits gained weight, as expected for young animals, during quarantine and after administration of DIGL-RP (Appendix D).

Dermal: Skin irritation signs are presented in Appendix E. Signs of erythema were observed in 11 of 12 rabbits. This erythema could not be attributed to the test compound because it occurred outside the patch site, or only occurred along the margin of the patch application area, or was associated with molting or clipper marks.

There were no gross findings in the rabbits at necropsy or microscopic findings of skin from selected areas of five rabbits that could be attributed to dermal exposure to DIGL-RP. A copy of the complete pathology report appears in Appendix F.

## DISCUSSION

Acute dermal toxicity testing is designed to evaluate both systemic toxicity due to percutaneous absorption of the test material and local toxicity from its contact with the skin. From these observations it can be determined whether absorption of the test material across the skin is sufficient to produce systemic effects or lethality. In the present study, no dermal reactions nor systemic effects attributable to dermal administration of DIGL-RP were observed.

All of the animals exposed to a limit dose of 2.0 g/kg DIGL-RP survived to the end of the test. None of these test animals exhibited any clinical signs suggestive of a systemic action by DIGL-RP. The only clinical signs observed during the study were slight diarrhea in five rabbits, nasal discharge in one rabbit, panting in all 12 rabbits, and lacrimation in one rabbit. The diarrhea occurred at the very beginning of the study in five animals and was considered a stress response to handling/clipping. A greenish-yellow nasal discharge is characteristic of rabbit Pasteurellosis (snuffles). These clinical signs were thus considered incidental to DIGL-RP treatment especially since they did not correlate with the pattern of acute toxicity observed following DIGL-RP administration to other species. In an acute oral toxicity study in rats, cyanosis and central nervous system-neuromuscular signs were the primary clinical signs associated with DIGL-RP administration (4). The lack of toxicity following dermal administration of DIGL-RP may be attributed to the fact that significant quantities of test compound remained on the back of the rabbits when the wrappings were removed after 24 hours of exposure.

Slight to mild erythema was observed initially after removal of the wrappings in 11 of the 12 dosed rabbits. However, this erythema could not be attributed to the test compound because it occurred outside the patch site, or only occurred along the margin of the patch application area (tape irritation), or was associated with molting or clipper marks (burns). This is consistent with the report that DIGL-RP was a non irritant in specialized dermal irritation studies (4).

This results of this study indicate that DIGL-RP has minimal potential to produce dermal irritation and is non-toxic when applied topically to the skin.

## CONCLUSION

A limit dose of 2.0 g/kg DIGL-RP was not lethal to rabbits nor did it produce significant systemic effects following dermal exposure for 24 hours. DIGL-RP Solid Propellant possesses a minimal potential for acute dermal toxicity.

## REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846.

2. Acute dermal toxicity study. LAIR Standard Operating Procedure OP-STX-30, Presidio of San Francisco, CA: Letterman Army Institute of Research, 18 May 1984.

3. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Acute exposure, dermal toxicity. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.

4. Frost DF, Brown LD, Morgan EW, Korte DW, Jr. Acute toxicity of diethyleneglycol dinitrate (DEGDN) and two DEGDN-containing solid propellants, DIGL-RP and JA-2. Laurel, MD: Chemical Propulsion Information Agency, 1988; CPIA Publication 485, p. 305-314.

## Appendix A: CHEMICAL DATA

Chemical Name: DIGL-RP Solid Propellant

LAIR Code Number: TP57

Physical State: Solid black cylinders (stick configuration)

Preparation of test substance for dosing: The cylinders of DIGL-RP were ground under liquid nitrogen using a Spex freezer mill. After grinding, the powder was sieved through an 80-mesh screen.

Chemical analysis:

DEGDN was the only major component of DIGL which could be easily analyzed. For analysis, samples of DIGL powder were added to individual 100 ml volumetric flasks.[1] After dilution to volume with 90% ethanol, a second 1:100 dilution was performed. These solutions were analyzed by HPLC. Standards consisted of solutions of DEGDN in ethanol, ranging in concentration from 164.5 to 670.5 µg/ml. Analysis of DEGDN by HPLC was performed under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm, Brownlee Labs, Inc., Santa Clara, CA); solvent system, 40% water - 60% acetonitrile); flow rate, 0.9 ml/min; wavelength monitored, 210 nm.[2] Under these conditions, DEGDN eluted with a retention time of approximately 5.4 min. The results from the analysis of standards and DIGL powder samples are presented in Tables 1 and 2.

Table 1. Analysis of Standards

| Concentration of Standard (µg/ml) | Peak Area* (x $10^{-7}$) |
|---|---|
| 164.5 | 0.94 |
| 191.0 | 1.09 |
| 275.5 | 1.60 |
| 299.4 | 1.74 |
| 362.0 | 2.08 |
| 399.6 | 2.31 |
| 444.4 | 2.52 |
| 539.8 | 3.07 |
| 585.0 | 3.32 |
| 670.5 | 3.79 |

*Average of 2 determinations

Equation for line by linear regression analysis:

$Y = 5.62 \times 10^4 X + 3.51 \times 10^5$, $r^2 = 0.9999$

## Appendix A (cont.): CHEMICAL DATA

Table 2. Analysis of DIGL Powder

| Weight of DIGL Analyzed (mg) | Dilution Factor | Peak Area (x $10^{-7}$) | Conc. of DEGDN in DIGL (weight %)* |
|---|---|---|---|
| 111.7 | 100 | 2.45 | 38.5 |
| 112.6 | 100 | 2.46 | 38.3 |
| 100.1 | 100 | 2.21 | 38.7 |

*Calculated using the equation for the standard curve as follows:
= {[Peak Area - 3.51 x $10^5$]/5.62 x $10^4$} + wgt DIGL (mg) x 10.

The average value for the concentration of DEGDN in DIGL was 38.5% and this agrees closely with the value of 36.70 ± 1.50 reported in the manufacturer's data sheet.

Stability:

The aqueous stability of the DEGDN component in the DIGL powder was examined.[3] The amount of DEGDN in aqueous DIGL suspensions was determined immediately after preparation of a suspension and again 24 hrs later. The study was conducted as follows. A suspension of DIGL in 1% gum tragacanth (200 mg/ml) was prepared. Three 1 ml aliquots were removed from the suspension immediately after preparation and again 24 hrs later. The 1 ml samples were transferred to individual 100 ml volumetric flasks. After diluting to volume with ethanol, the flasks were shaken well. A sample from each was analyzed by HPLC as described above. The average of the peak area values was 4.03 ± 0.12 for the 0 time samples and 4.10 ± 0.14 for the 24-hour samples. These results indicate that there was no decomposition of DEGDN in 1% gum tragacanth for a period of 24 hours.

Source: Radford Army Ammunition Plant, Radford, Virginia
(prime contractor: Hercules, Inc., Wilmington, Delaware)

Lot No.: RAD83M001S169

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1 Wheeler CW. Toxicity Testing of Propellents. Laboratory Notebook #85-12-023, p. 51-61. Letterman Army Institute of Research, Presidio of San Francisco, CA.

2 Wheeler CW. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p. 58. Letterman Army Institute of Research, Presidio of San Francisco, CA.

3 Wheeler CW. Toxicity Testing of Propellents. Laboratory Notebook #85-12-023, p. 24-42. Letterman Army Institute of Research, Presidio of San Francisco, CA.

## Appendix A (cont.): CHEMICAL ANALYSIS

### Manufacturer's Data Sheet for DIGL-RP Formulation

| Ingredients | Finished Propellant Percentage |
|---|---|
| Nitrocellulose (13.05 ±0.05% Nitrogen) (6-12 seconds viscosisty) | 62.5 ±2.00 |
| Diethyleneglycol Dinitrate (DEGDN) | 36.70 ±1.50 |
| Ethyl Centralite (EC) | 0.25<br>0.25 ±0.05 |
| Akardit II | 0.25<br>0.45 ±0.15 |
| Magnesium Oxide | 0.05 Max |
| Graphite (Chg 5) | 0.05 Max |
| | 100.00 |

## Appendix B: ANIMAL DATA

Species: *Oryctolagus cuniculus*

Strain: New Zealand White (albino)

Source: Elkhorn Rabbitry
5265 Starr Way
Watsonville, CA 95076

Sex: Male and female

Age: Date of birth - 30 Aug 85

Animals in each group: 6 males and 6 females

Condition of animals at start of study: Normal

Body weight range at dosing: 2.5 - 3.1 kg

Identification procedures: Ear tattoo.

Pretest conditioning:

1. Quarantine/acclimation period from 14 Nov - 3 Dec 1985.
2. Animals were close-clipped and examined 24 hours before dosing

Justification:

The laboratory rabbit is a proven mammalian model for dermal toxicity studies because of its size, ease of restraint, and skin permeability.

## Appendix C: HISTORICAL LISTING OF STUDY EVENTS

| DATE | EVENT |
|---|---|
| 14 Nov 85 | Thirteen rabbits arrived at LAIR. They were checked for illness and quarantined in the Division of Animal Care and Services. |
| 15 - 26 Nov 85 | Animals were observed daily. |
| 15, 22, 27 Nov<br>3, 10, 18 Dec 85 | Rabbits were weighed. |
| 18 Nov 85 | Rabbits were tattooed on the ear and treated prophylactically for coccidia and ear mites. One female was submitted for quality control necropsy. |
| 27 Nov 85 | Rabbits were transferred to Toxicology Suite. |
| 27 Nov - 3 Dec 85 | Rabbits were checked daily for illness. |
| 2 Dec 85 | Rabbits were close-clipped and examined. |
| 3 Dec 85 | Rabbits were close-clipped. |
| 4 Dec 85 | Twelve rabbits were dosed. Observations and clinical signs were recorded 3 times (2, 4, and 5 hours after dosing). |
| 5 Dec 85 | Wrappings were removed and rabbits were observed for dermal and clinical signs of toxicity. |
| 5 - 18 Dec 85 | 1/2-hour and daily dermal scorings were performed. |
| 5 - 18 Dec 85 | Rabbits were observed in the morning for clinical signs. A walk-through check was performed in the afternoon. |
| 18 Dec 85 | Rabbits were submitted for necropsy Skin from exposure and control sites on five rabbits was preserved for histological examination. |

## Appendix D: BODY WEIGHT DATA

| Animal Number | Day 01 | 08 | 013 | 019 | 6 | 14 |
|---|---|---|---|---|---|---|
| | | | Females | | | |
| 85F288 | 2445 | 2640 | 2586 | 2735 | 2796 | 2945 |
| 85F289 | 2250 | 2485 | 2343 | 2575 | 2671 | 2935 |
| 85F290 | 2280 | 2470 | 2338 | 2578 | 2745 | 2948 |
| 85F291 | 2060 | 2410 | 2317 | 2508 | 2705 | 2861 |
| 85F293 | 2660 | 2815 | 2625 | 2747 | 2774 | 2865 |
| 85F294 | 2670 | 2765 | 2725 | 3015 | 3191 | 3202 |
| Mean | 2394 | 2598 | 2489 | 2693 | 2814 | 2959 |
| Standard Error | ±99 | ±69 | ±72 | ±75 | ±78 | ±51 |
| | | | Males | | | |
| 85F295 | 2430 | 2525 | 2479 | 2713 | 2863 | 2965 |
| 85F296 | 2570 | 2870 | 2731 | 3050 | 3060 | 3155 |
| 85F297 | 2655 | 2785 | 2699 | 2954 | 3064 | 3156 |
| 85F298 | 2410 | 2700 | 2590 | 2880 | 3002 | 3217 |
| 85F299 | 2425 | 2575 | 2531 | 2808 | 2920 | 3045 |
| 85F300 | 2065 | 2360 | 2342 | 2570 | 2595 | 2736 |
| Mean | 2426 | 2636 | 2562 | 2829 | 2917 | 3046 |
| Standard Error | ±82 | ±76 | ±59 | ±70 | ±72 | ±72 |

* Weights are given in grams.

## Appendix E: INDIVIDUAL DERMAL SIGNS

| Animal Number | Dermal Signs | Duration of Dermal Signs(Days) | Severity* | Area† |
|---|---|---|---|---|
| | | **Females** | | |
| 85F288 | Erythema | 1-4 | A | 2-3 |
| 85F289 | Erythema | 1-4 | A | 2-3 |
| 85F290 | Erythema | 1 | B | 3 |
| 85F291 | Erythema | 1,8 | A | 1-2 |
| 85F293 | Erythema | 1 | B | 4 |
| 85F294 | Erythema | 1,8-13 | A | 1,3 |
| | | **Males** | | |
| 85F295 | Erythema | 1,8-13 | A | 1-2 |
| 85F296 | Erythema | 1 | A | 2 |
| 85F297 | None | N/A | N/A | N/A |
| 85F298 | Erythema | 1-4,8 | A-B | 1-3 |
| 85F299 | Erythema | 1 | A | 1 |
| 85F300 | Erythema | 1-8 | A-B | 1-2 |

* Severity Scores: A = Slight
B = Mild
C = Moderate
D = Severe

† Pertains to percent of exposed area exhibiting signs of dermal irritation. This value is determined by visual approximation.

1 = 5%
2 = > 5 to 10%
3 = >10 to 25%
4 = >25 to 50%
5 = >50%

## Appendix F: PATHOLOGY REPORT

Pathology Report
GLP Study 85024
Acute Dermal Toxicity Test

Investigator: MAJ Brown

Substance: DIGL-RP

Species: Rabbit, NZW, 6 male, 5 female approximately 4 months old.

History: See LAIR SOP-OP-STX-30. All animals were killed by exsanguination following sodium pentobarbital anesthesia.

Gross Necropsy Findings:

| LAIR ACC# | ANIMAL ID# | SEX | DIAGONSIS |
|---|---|---|---|
| 38729 | 85F288 | F | Not remarkable (NR) |
| 38730 | 85F289 | F | NR |
| 38731 | 85F290 | F | NR |
| 38732 | 85F291 | F | NR |
| 38733 | 85F293 | F | NR |
| 38734 | 85F294 | F | NR |
| 38735 | 85F295 | M | NR |
| 38736 | 85F296 | M | NR |
| 38737 | 85F297 | M | Purulent otitis media, right ear |
| 38738 | 85F298 | M | NR |
| 38739 | 85F299 | M | NR |
| 38740 | 85F300 | M | Liver - focal 1.5 cm rough granular surface |

## Appendix F (cont): PATHOLOGY REPORT

Pathology Report
GLP Study 85024

Microscopic Findings: Skin sections control and treated.

38729 - 1 (treated): Not remarkable (NR)
38729 - 2 (control): NR

38730 - 1: NR
38730 - 2: NR

38735 - 1: Dermatitis, histiocytic, heterophilic, subacute, multifocal, minimal.
38735 - 2: NR

38736 - 1: NR
38736 - 2: NR

38740 - 1 Liver: Cholangiohepatitis, granulomatous, fibrosing, chronic, portal with bridging, marked.
38740 - 2 Liver: NR

Comment: The gross lesions were considered incidental findings and not related to the treatment. No microscopic evidence of dermal toxicity was seen.

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